Anti-human IL-12 Neutralizing Antibody

ORDERING INFORMATION

Catalog Number: AB-219-NA

Lot Number: EW03

Size: 1 mg

Formulation: sterile solution in PBS

Storage: -20° C

Reconstitution: sterile PBS

Specificity: rhIL-12

Immunogen: *Sf 21*-derived rhIL-12

Ig class: Total goat IgG

Applications: Neutralization of bioactivity
Western blot
ELISA

Preparation

Produced in goats immunized with purified, insect cell line *Sf* 21-derived, recombinant human interleukin 12 (rhIL-12). Total IgG was purified by Protein G affinity chromatography.

Formulation

Lyophilized from a 0.2 µm sterile-filtered solution in phosphate-buffered saline (PBS).

Endotoxin Level

< 10 ng per 1 mg of the antibody as determined by the LAL method.

Reconstitution

Reconstitute with sterile PBS. If 1 mL of PBS is used, the antibody concentration will be 1 mg/mL.

Storage

Lyophilized samples are stable for greater than 6 months at -20° C to -70° C. Reconstituted antibody is stable for at least 1 month at 2° - 4° C or 3 months at -20° to -70° C under sterile conditions. **Avoid repeated freeze-thaw cycles.**

Specificity

This antibody has been selected for its ability to neutralize the biological activity of rhIL-12. Based on direct ELISA and western blot results, this antibody shows less than 2% cross-reactivity with rmIL-12. Additionally, in direct ELISA, this antibody shows no cross-reactivity with other cytokines tested.[1]

Neutralization of Human IL-12 Bioactivity

The exact concentration of antibody required to neutralize IL-12 activity is dependent on the cytokine concentration, cell type, growth conditions and the type of activity studied. To provide a guideline, R&D Systems has determined the neutralization dose for this antibody under a specific set of conditions. The **Neutralization Dose$_{50}$ (ND$_{50}$)** for this antibody is defined as that concentration of antibody required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when that cytokine is present at a concentration just high enough to elicit a maximum response.

As shown in figures 1 and 2 on the next page, the ND$_{50}$ for this lot of anti-human IL-12 antibody was determined to be approximately 1.0 - 2.0 µg/mL in the presence of 1.0 ng/mL of rhIL-12, using PHA activated human peripheral blood mononuclear cells (PBMC). The specific conditions are described in the figure legends.

Additional Applications

For direct ELISAs, the antibody can be used at 0.5 - 1.0 µg/mL with the appropriate secondary reagents to detect IL-12. The detection limit for rhIL-12 is approximately 0.6 ng/well.

For western blot analysis, the antibody can be used at 1 - 2 µg/mL with the appropriate secondary reagents to detect IL-12. The detection limit for rhIL-12 is approximately 20.0 ng/lane under non-reducing and reducing conditions. Under reducing conditions, both the p35 and p40 subunits of IL-12 are detected. Because this antibody preparation is a total IgG fraction, complete monospecificity cannot be assumed.

Figure 1

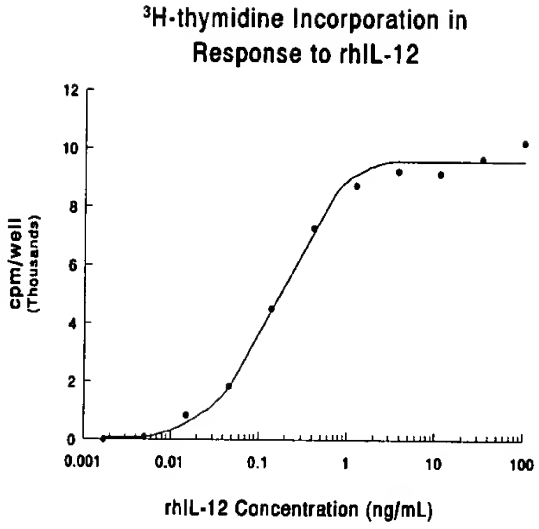

Figure 2

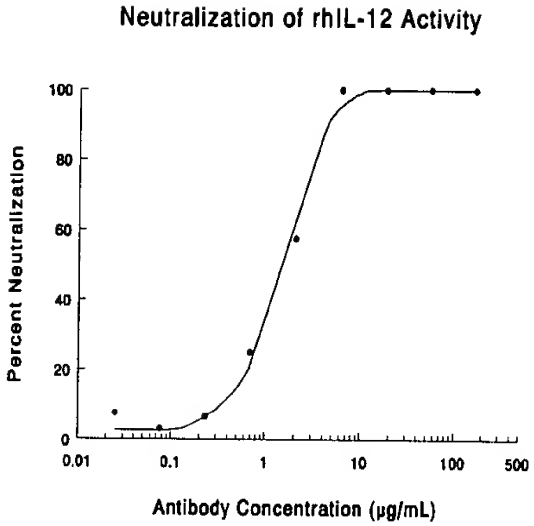

Figure 1
Human IL-12 stimulates the ^{3}H-thymidine incorporation by PHA activated human peripheral blood mononuclear cells in a dose-dependent manner (Yokota, T. *et al.*, 1986, Proc. Natl. Acad. Sci. USA **83**:5894). The ED_{50} for this effect is typically 0.1 - 0.2 ng/mL.

Figure 2
To measure the ability of the antibody to neutralize the bioactivity of rhIL-12 on activated PBMNCs, rhIL-12 was incubated with various concentrations of the antibody for 1 hour at 37° C in a 96 well microtiter plate. Following this preincubation period, PBMNCs were added. The assay mixture in a total volume of 100 µL, containing antibody at the concentrations indicated, rhIL-12 at 1.0 ng/mL and cells at 2 x 10^5 cells/mL, was incubated at 37° C for 48 hours in a humidified CO_2 incubator. ^{3}H-thymidine was added during the final 4 hours of incubation. The cells were harvested onto glass fiber filters and the ^{3}H-thymidine incorporated into DNA was determined. The ND_{50} of the antibody is approximately 1.0 - 2.0 µg/mL.

[1]rhANG, rhβ-NGF, rhCNTF, rhEGF, rhEPO, rhFGF acidic, rhFGF basic, rhFGF-4, rhFGF-5, rhFGF-6, rhG-CSF, rhGM-CSF, rmGM-CSF, rhGROα, rhHGF, rhIFN-γ, rhIGF-I, rhIGF-II, rhIL-1α, rmIL-1α, rhIL-1β, rmIL-1β, rhIL-1ra, rhIL-2, rhIL-2 Rα, rhIL-3, rhIL-3 Rα, rmIL-3, rhIL-4, rhIL-4 Rα, rmIL-4, rhIL-5, rhIL-5 Rα, rmIL-5, rhIL-6, rhIL-6 sR, rmIL-6, rhIL-7, rmIL-7, rhIL-8, rhIL-9, rmIL-9, rhIL-10, rmIL-10, rhIL-11, rhLIF, rmLIF, rhM-CSF, rhMCP-1, rhMIP-1α, rmMIP-1α, rhMIP-1β, rmMIP-1β, rhOSM, hPDGF, pPDGF, rhPDGF-AA, rhPDGF-AB, rhPDGF-BB, rhPTN, rhRANTES, rhSCF, rmSCF, rhSLPI, rhTGF-α, rhTGF-β1, pTGF-β1.2, pTGF-β2, rcTGF-β3, raTGF-β5, rhLAP (TGF-β1), rhLatent TGF-β1, rhTGF-β sRII, rhTNF-α, rmTNF-α, rhTNF-β, rhsTNF RI, rhsTNF RII, rhVEGF